APCRA Prospective Study

Assessment of chemicals, using and developing New Approach Methodologies (NAMs)

Mark Viant, Tomasz Sobanski &
Case Study Team

4th APCRA meeting, 9-10 October 2019

ECHA
EUROPEAN CHEMICALS AGENCY

Disclaimer

The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of any participating government organization.

Project partners

Lit-Hsin Loo, Peiying Chuan

Bioinformatics Institute, Agency for Science, Technology and Research, Singapore (A*STAR)

Marc Beal, Matthew Gagné, Tara Barton-Maclaren

Healthy Environments and Consumer Safety Branch, Health Canada, Government of Canada

Katie Paul Friedman, Maureen Gwinn, Russell Thomas

Center for Computational Toxicology & Exposure, Office of Research and Development, US Environmental Protection Agency (CCTE)

John Bucher, Scott Masten

National Toxicology Program, National Institute of Environmental Health Sciences, National Institutes of Health (NTP)

Lidka Maslankiewicz, Joop De Knecht

Netherlands National Institute for Public Health and the Environment (RIVM)

Daniel Ta-Jen Chang

Office of Chemical Safety and Pollution Prevention,

US Environmental Protection Agency

John Colbourne, Mark Viant

Michabo Health Science, University of Birmingham Enterprise

Maurice Whelan

Systems Toxicology Unit, Joint Research Centre, European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM), Ispra, Italy

Karin Kilian

Sustainable Chemicals Unit, DG Environment, European Commission

ECHA
EUROPEAN CHEMICALS AGENCY

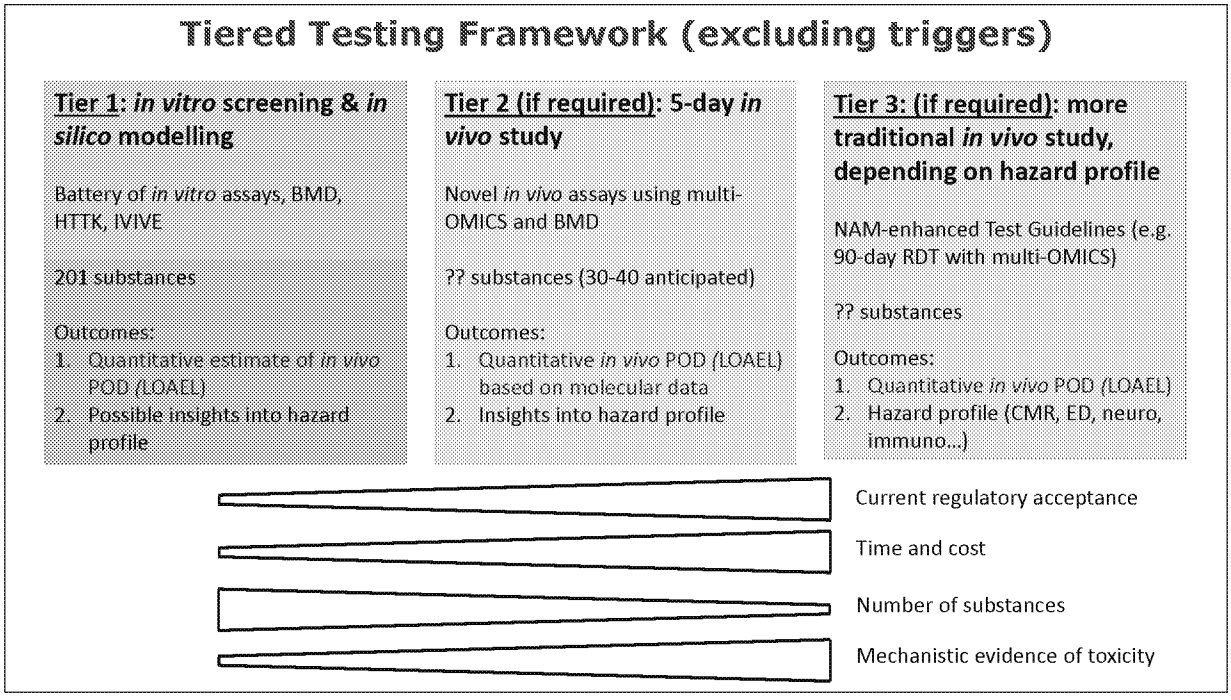
Core Objectives

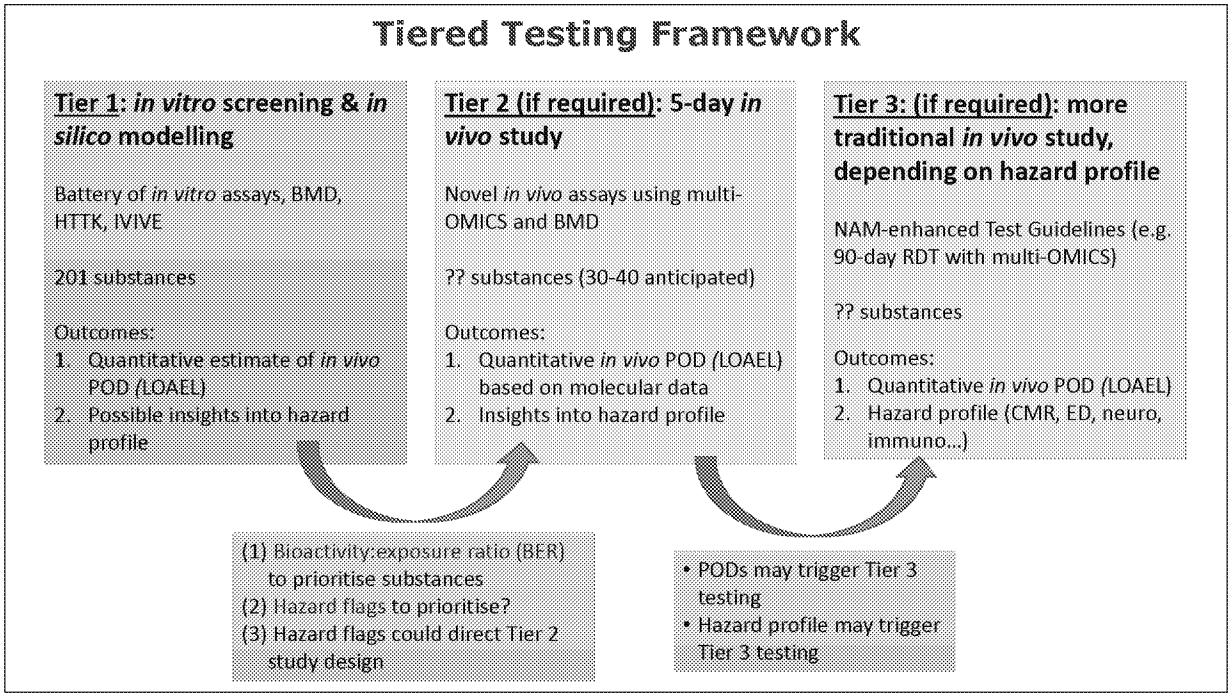
- To implement and evaluate a 3-tiered workflow that comprises of multiple NAMs (including a Tier 1 battery of *in vitro* tests, OMICS, BMD to derive PODs, HTTK, IVIVE) to screen, prioritise and, where needed, more deeply risk assess chemicals (including 5-day *in vivo* Tier 2 tests)…
- … in order to assess whether NAMs can provide a conservative *in vivo* point of departure / LOAEL for regulatory risk assessment.

ECHA
EUROPEAN CHEMICALS AGENCY

Other Objectives

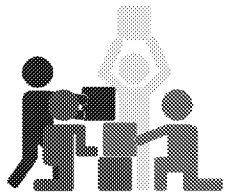
- To demonstrate this approach for data poor industrial chemicals.
- To assess chemicals in an international context.
- Confidence building in application of NAMs for hazard characterisation.


Tiered Testing Framework (excluding triggers)
Tier 1: in vitro screening & in silico modelling
Battery of in vitro assays, BMD, HTTK, IVIVE
201 substances
Outcomes:
1. Quantitative estimate of in vivo POD (LOAEL)
2. Possible insights into hazard profile
Tier 2 (if required): 5-day in vivo study
Novel in vivo assays using multi-OMICS and BMD
?? substances (30-40 anticipated)
Outcomes:
1. Quantitative in vivo POD (LOAEL) based on molecular data
2. Insights into hazard profile
Tier 3: (if required): more traditional in vivo study, depending on hazard profile
NAM-enhanced Test Guidelines (e.g. 90-day RDT with multi-OMICS)
?? substances
Outcomes:
1. Quantitative in vivo POD (LOAEL)
2. Hazard profile (CMR, ED, neuro, immuno…)
Current regulatory acceptance
Time and cost
Number of substances
Mechanistic evidence of toxicity


Tiered Testing Framework
Tier 1: in vitro screening & in silico modelling
Battery of in vitro assays, BMD, HTTK, IVIVE
201 substances
Outcomes:
1. Quantitative estimate of in vivo POD (LOAEL)
2. Possible insights into hazard profile
Tier 2 (if required): 5-day in vivo study
Novel in vivo assays using multi-OMICS and BMD
?? substances (30-40 anticipated)
Outcomes:
1. Quantitative in vivo POD (LOAEL) based on molecular data
2. Insights into hazard profile
Tier 3: (if required): more traditional in vivo study, depending on hazard profile
NAM-enhanced Test Guidelines (e.g. 90-day RDT with multi-OMICS)
?? substances
Outcomes:
1. Quantitative in vivo POD (LOAEL)
2. Hazard profile (CMR, ED, neuro, immuno…)
(1) Bioactivity:exposure ratio (BER) to prioritise substances
(2) Hazard flags to prioritise?
(3) Hazard flags could direct Tier 2 study design
• PODs may trigger Tier 3 testing
• Hazard profile may trigger Tier 3 testing

Original timeline

- Substance selection
  Q3 2017 - Q3 2018 (*finished*)
- Tier 1 *in vitro* testing & *in silico* modelling
  Q4 2018 - Q2 2020 (*nearing completion*)
- Tiers 2 and 3 *in vivo* testing
  Q3 2019 - Q4 ~~2020~~ 2021 (*preparatory work is ongoing*)
- Analysis of the results & communication
  Q1 2019 - Q4 2021 (*on-going*)



ECHA
EUROPEAN CHEMICALS AGENCY

Substance selection

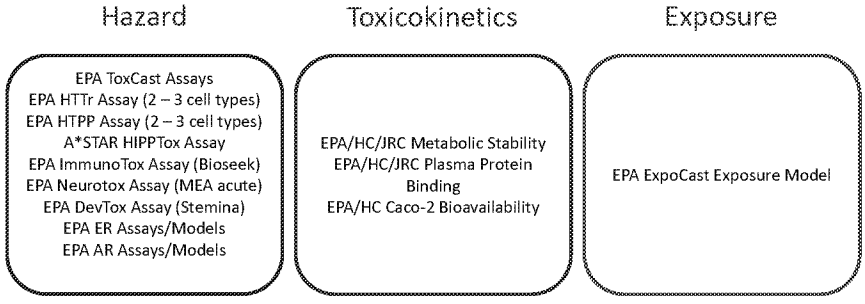
- Scenario 1: substance present on the EU and/or Canada and/or US market, with a potential for consumer use and significant data gaps for systemic toxicity (105).
- Scenario 2: substance present on the EU and/or Canada and/or US market, with known toxicity or with the potential to exhibit different toxicity levels across different species (8).
- Scenario 3: substances selected from APCRA retrospective study (88).

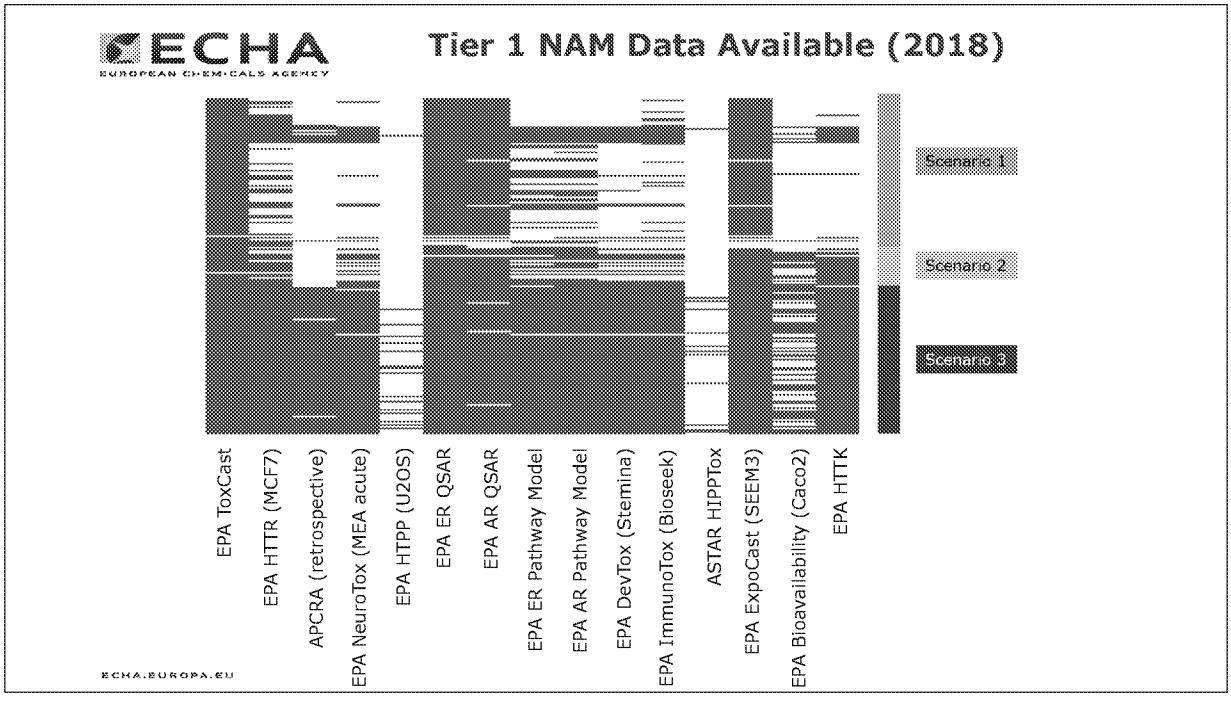
Scenario 3 substances were selected from the following groups:

- with POD_{nam} estimates that are less conservative than $POD_{traditional}$
- with POD_{nam} estimates close to $POD_{traditional}$
- with an overly conservative POD_{nam} estimate.

ECHA
EUROPEAN CHEMICALS AGENCY

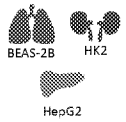
Tier 1 Battery of Assays / Models




ECHA
EUROPEAN CHEMICALS AGENCY
Tier 1 NAM Data Available (2018)
Scenario 1
Scenario 2
Scenario 3
EPA ToxCast
EPA HTTR (MCF7)
APCRA (retrospective)
EPA NeuroTox (MEA acute)
EPA HTPP (U2OS)
EPA ER QSAR
EPA AR QSAR
EPA ER Pathway Model
EPA AR Pathway Model
EPA DevTox (Stemina)
EPA ImmunoTox (Bioseek)
ASTAR HIPPTox
EPA ExpoCast (SEEM3)
EPA Bioavailability (Caco2)
EPA HTTK
ECHA.EUROPA.EU

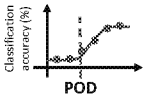
NAM Data Available from A*STAR, Singapore (2019)

201 Selected Chemicals (by APCRA partners)
[Received 199 chemicals, 100% Done, Nov 2018]



Single-concentration Cell Viability Check
[100% Done, June 2019]

Multi-concentration Phenotypic Profiling (HRPFlux)
[48% Done, on-going]



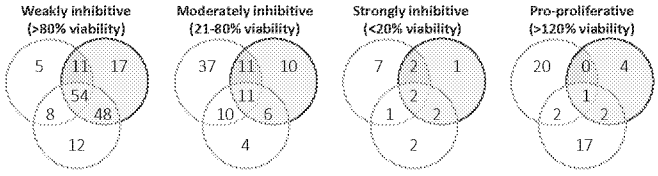
Points of Departure (POD) Estimation
[By Dec 2019]

ToxCast PODs

Final in vitro POD derivation

Selection of chemicals for higher tier *in vivo* tests

Preliminary findings on single-concentration tests:



- ~73% (145) of the compounds are moderately or strongly inhibitive in at least one cell model
- Interestingly, ~23% (46) of the chemicals are pro-proliferative in at least one cell model

Tier 1 *in vitro* NAM data generation efforts (2019)

Data type	Contractor	Status
Immunotoxicity (inflammation)	Bioseek (Keith Houck)	• Chemicals to be received at Bioseek Sept 2019. • Data returned for pipelining within about 4 months of receipt.
Developmental toxicity	Stemina (Tom Knudsen)	• Chemicals to be received at Stemina Sept 2019; • Single concentration data to be returned Dec 2019; • Multi-conc screening in 2020.
Acute neurotoxicity	Microelectrode arrays with primary neurons (Tim Shafer)	• Data generated and to be shared for pipelining in Oct-Dec 2019
High-throughput phenotypic profiling	NCCT (Josh Harrill and Johanna Nyffeler) A*STAR	• NCCT: Complete for U-2 OS cells. • NCCT: Second cell type anticipated to be screened in 2020. • A*STAR: BEAS-2B, HK2, HepG2 by Dec 2019.
High-throughput transcriptomics	NCCT (Josh Harrill and Johanna Nyffeler)	• Complete for MCF-7 cells; data analysis in-process. • Complete screening for U-2 OS in late 2019. • Complete screening for HepaRG-2D in late 2019.
HTTK	JRC (Thomas Cole) HC (Marc Beal) NCCT (Barbara Wetmore and John Wambaugh)	• JRC contract was successful for most of the (~50) chemicals on their list; awaiting contractor report and then NCCT to process into the httk database/R package • HC contract successful for the 17 planned substances; data to be shared soon • NCCT: out of 7 remaining chemicals, 4 are scheduled to be tested by Barbara in late 2019/early 2020.
CaCo2 bioavailability	NCCT (John Wambaugh)	• QSAR model data expected 2020

... by end of Q2 2020

ECHA
EUROPEAN CHEMICALS AGENCY

Tier 2 *in vivo* testing: 5-day OMICS study

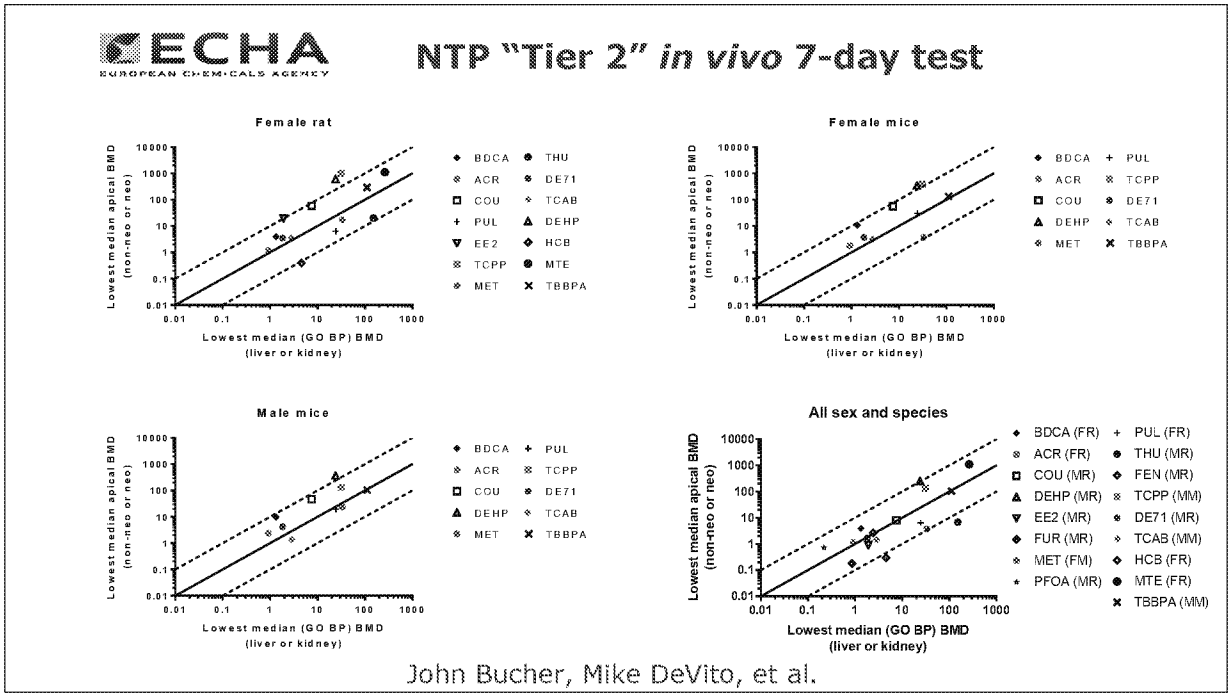
- NTP is currently demonstrating the use of short-term, 5-day *in vivo* assays which use transcriptomics as an alternative data stream for understanding hazard.
- Represents a bridge (requiring fewer animals) between HT *in vitro* assays (Tier 1) and traditional apical endpoints (Tier 3).
- Maybe able provide a rapid estimate of POD for traditional apical endpoints, and provide a broad screen of interpretable biological activity of test chemicals.

Thomas *et al.* study showed that…

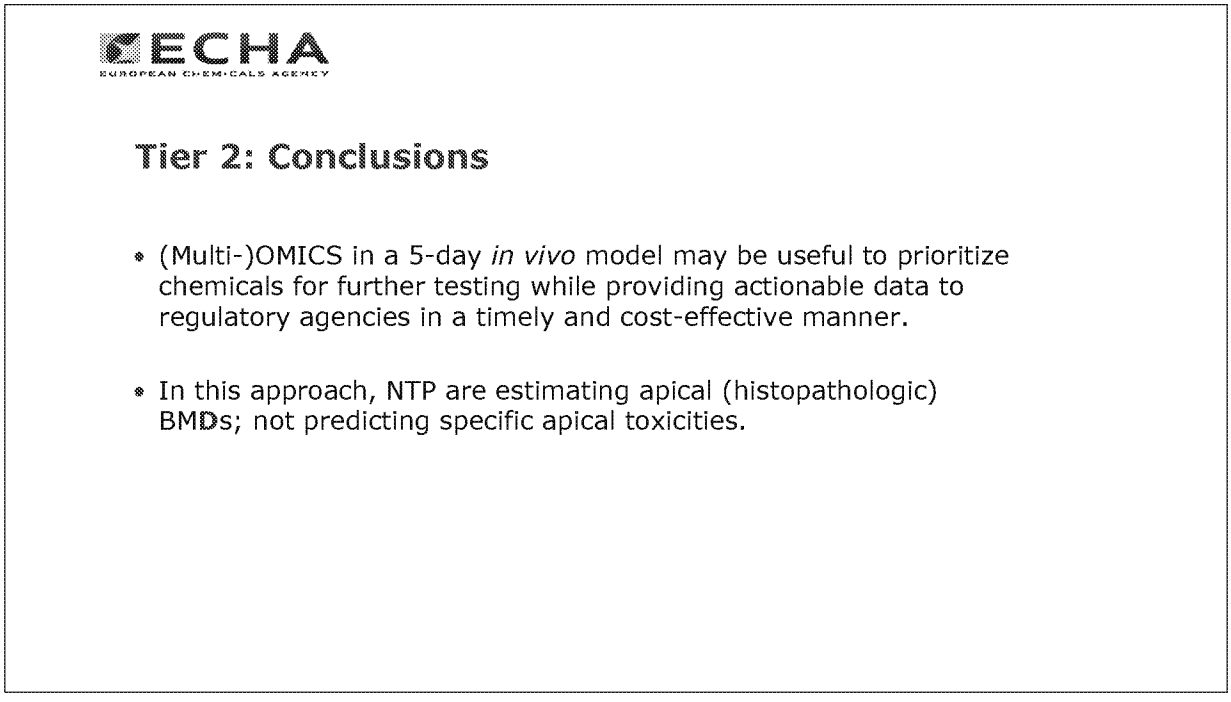
- The lowest transcriptional BMDs in specific target tissues (bladder, liver, and thyroid) correlated well with the lowest non-cancer apical BMDs in the **same target tissues** after 5 days of exposure **in rats**.

NTP's current study…

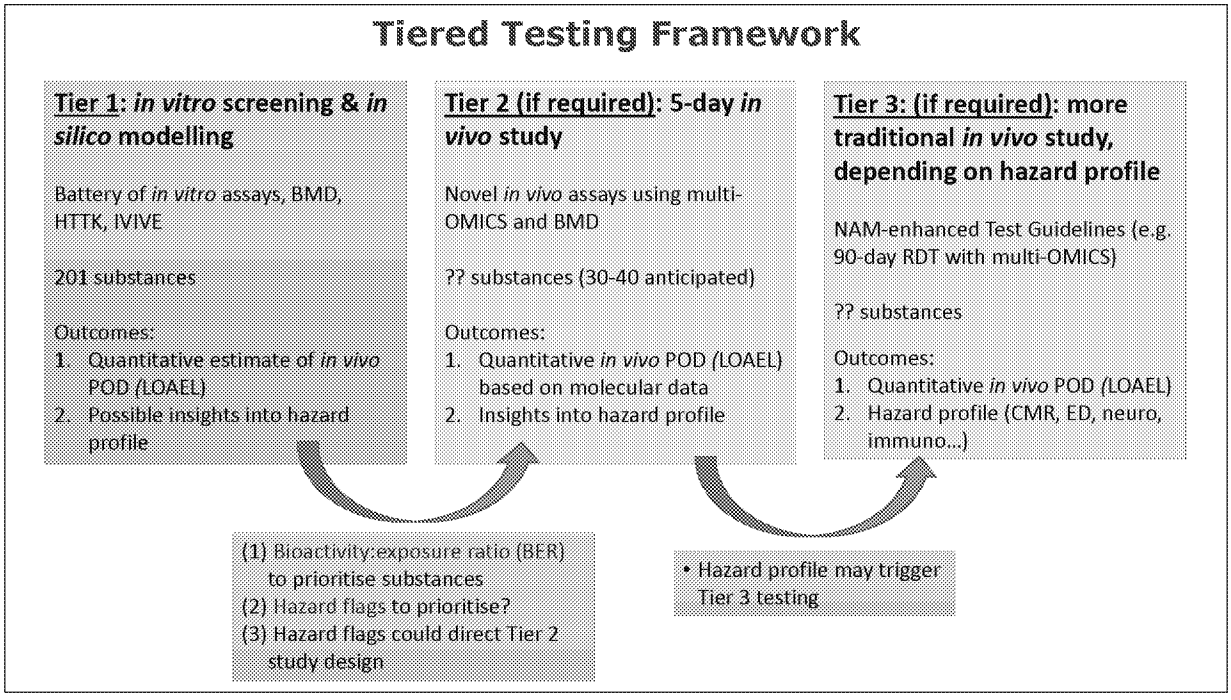
- Evaluates whether the lowest transcriptional BMDs in **liver and kidney (as 'sentinel' tissues)** after 5 days of exposure in **male rats** correlate with the lowest apical BMDs **in male and female rats and mice** from long-term (chronic or sub-chronic) toxicity studies.



Not all of the chemicals were tested in mice or female rats



MOE = POD/predicted or estimated dose of human exposure


Tiered Testing Framework
Tier 1: in vitro screening & in silico modelling
Battery of in vitro assays, BMD, HTTK, IVIVE
201 substances
Outcomes:
1. Quantitative estimate of in vivo POD (LOAEL)
2. Possible insights into hazard profile
Tier 2 (if required): 5-day in vivo study
Novel in vivo assays using multi-OMICS and BMD
?? substances (30-40 anticipated)
Outcomes:
1. Quantitative in vivo POD (LOAEL) based on molecular data
2. Insights into hazard profile
Tier 3: (if required): more traditional in vivo study, depending on hazard profile
NAM-enhanced Test Guidelines (e.g. 90-day RDT with multi-OMICS)
?? substances
Outcomes:
1. Quantitative in vivo POD (LOAEL)
2. Hazard profile (CMR, ED, neuro, immuno…)
(1) Bioactivity:exposure ratio (BER) to prioritise substances
(2) Hazard flags to prioritise?
(3) Hazard flags could direct Tier 2 study design
• Hazard profile may trigger Tier 3 testing

ECHA
EUROPEAN CHEMICALS AGENCY

Fast Tracking – "Early selection" of Candidates for Tier 2 Testing

- Fast tracking due to informative results from NTP's 5-day *in vivo* studies, and to begin to evaluate this Tier 2 testing in the APCRA Prospective study before completion of Tier 1 testing.

- Selection focused on:
  - BER < 10,000 (or, $\log_{10}$BER < 4)
  - Data-poor substance (lack of 90-day study)
  - Evidence for *in vitro* bioactivity
  - Planned hazard flags: endocrine activity; developmental toxicity; acute neurotoxicity; inflammation/immune response

ECHA
EUROPEAN CHEMICALS AGENCY

Conclusions

- Objectives and tiered testing framework for Prospective Study have been revisited and updated.
- Generation of extensive Tier 1 datasets progressing well, international effort!!
- 5-day *in vivo* OMICS studies are estimating apical (histopathologic) BMDs well, this new test design represents an excellent choice for Tier 2 testing.
- Proposal to fast-track substances to Tier 2 is helping to define the triggers from Tiers 1 to 2.